

# Protocol for Using the miniSTR System "miniNC01" on the ABI 3100 Instrument

## Reagents Included:

- MiniNC01 (Non-Codis) Primer mix, 100 reactions per tube + 10 % overfill (**1 green topped tube**). Approximately 550  $\mu\text{L}$ , 1  $\mu\text{M}$  conc.
- Allelic ladders, 20  $\mu\text{L}$  (**1 yellow topped tube**)

## Materials Needed:

- PCR mix from any STR kit (e.g. SGM Plus PCR mix)
- TaqGold DNA polymerase (5 U/ $\mu\text{L}$ )
- Capillary Array
- POP-6 or POP-4 polymer
- Matrix standards for ABI 3100
- Genetic Analyzer Buffer
- GeneScan and Genotyper software programs

## Primer Sequences (Coble and Butler, JFS, in press)\*

Locus		MiniNC01 Primer Sequences (5'-3')	Distance 3'end from STR repeat
D10S1248	F	<b>6FAM</b> -TTAATGAATTGAACAAATGAGTGAG	1
	R	GCAACTCTGGTTGTATTGTCTTCAT	0
D14S1434	F	<b>VIC</b> -TGTAATAACTCTACGA <b>CTGCTGCTCTG</b>	-11
	R	GAATAGGAGGTGGATGGATGG	0
D22S1045	F	<b>NED</b> -ATTTTCCCCGATGATAGTAGTCT	3
	R	GCGAATGTATGATTGGCAATATTTT	6

\*A PDF copy of this paper can be downloaded at the STRBase website:

<http://www.cstl.nist.gov/biotech/strbase/miniSTR/CobleandButlerJFS.pdf>

\*\*The 5' Guanine residue in each reverse primer (indicated in bold, red font) was added to promote adenylation

\*\*\*The negative number of nucleotide bases from the STR repeat in D14S1434 indicates that the end of the primer is in the repeat region (with the nucleotides in red italics at the 3' end of the primer).

## Comments, suggestions, issues, notes for user...

- In our hands, this assay is sensitive to ~20 pg DNA at 32 cycles of PCR.

- Information for detection of PCR products using an ABI 310 can be found in the Butler et al. (2003) reference above, or can be sent to you via e-mail (see contact info below).

- Observed heterozygosities (n=474 genotypes) for the markers in miniNC01 are: D10S1248, 0.78; D14S1434, 0.68; D22S1045, 0.77.

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## PCR Conditions:

### Preparation of Master Mix:

\_\_\_ (# reactions) x **10.5  $\mu\text{L}$  PCR mix (from an ABI kit)** = \_\_\_\_\_

\_\_\_ (# reactions) x **0.4  $\mu\text{L}$  Taq Gold** = \_\_\_\_\_

\_\_\_ (# reactions) x **5.5  $\mu\text{L}$  primer mix (green-topped tube)** = \_\_\_\_\_  
**16.4  $\mu\text{L}$  – includes overfill for pipetting**

### Preparation of Individual PCR Reactions:

**15  $\mu\text{L}$  master mix (from above)**

**10  $\mu\text{L}$  DNA template** (or dI H<sub>2</sub>O to bring up the volume)

### Thermal Cycling

Thermal cycling was performed with the GeneAmp 9700 (Applied Biosystems) using the following conditions in 9600-emulation mode (i.e., ramp speeds of 1 °C/s):

95 °C for 10 minutes

**32 cycles:** 94 °C for 1 minute

55 °C for 1 minute

72 °C for 1 minute

60 °C for 45 minutes

25 °C forever

## Detection of PCR Products

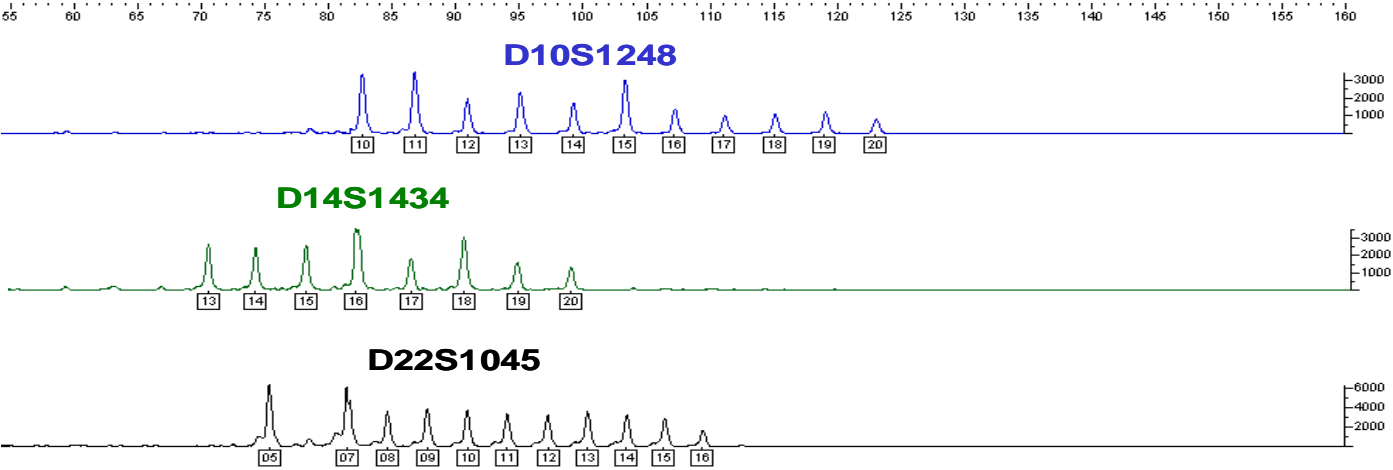
We have used the ABI 3100 with POP6 polymer, capillaries, and buffer used for STR typing with commercial kits. The MiniNC01 assay uses 6FAM (blue), VIC (green), NED (yellow) dyes.

### ABI 3100

Prior to running any samples with the MiniNC01 STR system on the ABI 3100, a 5 dye matrix needs to be established under the "G5 filter" with the dyes 6FAM (blue), VIC (green), NED (yellow), PET (red), and LIZ (orange) using **matrix standard set DS-33** (P/N 4318159). Samples are typically prepared with **15  $\mu\text{L}$  Hi-Di™ formamide** (Applied Biosystems, P/N 4311320), **0.35  $\mu\text{L}$  GS500 LIZ** (P/N 4322682), and with **1  $\mu\text{L}$  PCR product**.

The samples may be run using the default module **GeneScan36\_POP4DefaultModule**, which performs an electrokinetic injection onto the 16-capillary array for 10 s at 3,000 volts. The STR alleles are then separated at 15,000 volts for approximately 30 minutes with a run temperature of 60 °C using the 3100 POP™-6 sieving polymer (Applied Biosystems, P/N 4316355), 1X Genetic Analyzer Buffer with EDTA (P/N 402824), and a 36 cm array (P/N 4315931).

Allelic Ladders with miniNC01 markers



Expected Control Results		
STR Locus	Control DNA 007 Genotype	Control DNA 9947A Genotype
D10S1248	13, 16	14, 16
D14S1434	15, 18	15, 17
D22S1045	8, 13	8, 11

Macro Information:

A downloadable research macro using fixed bins will be posted for you on the STRBase website:  
<http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm>

Also posted is a document with allele size ranges for each bin (for those wanting to build their own macro).

Negative control from sensitivity series using the suggested conditions - 32 cycles, 2U Taq

